

II. SUMMARY OF THE INVENTION

[illegible]

[0035] According to another feature of the present invention, there is provided a method for assaying, or determining the presence or amount of an analyte in a test sample by: Monitoring an analyte-mediated ligand binding event in a test mixture by forming a test mixture comprising the test sample, a labeled analyte-analog and at

ing member immobilized on a particles bearing a surface capable of inducing surface-enhanced Raman light scattering wherein the labeled enzyme-antibody complex as an enzyme-binding molecule expressing an enzyme epitope, is recognized by a specific binding member, said enzyme-binding being attached to a Raman-active label.

wherein the extent of the blocking of the labeled analyte bound to the specific binding member on the particulate is affected by the presence of the analyte. Then, illuminating the test mixture with a radiation sufficient to cause

According to another feature of the present invention, the amount of the surface-etched layer is being determined upon the amount of the same.

forming the presence or amount of an analyte in a test sample by monitoring an enzyme-mediated signal produced in a test mixture by forming the test mixture from the test sample containing the analyte and a particular substrate capable of reacting with the enzyme to produce a signal. The signal is then measured and compared to a particular value having a surface concentration of the analyte.

whereas the chromatographic material comprises a capillary tube having associated with it a flame active label, then applying the test mixture onto a chromatographic material having a proximal and a distal end wherein the chromatographic material comprises a capillary tube having associated with it a flame active label.

[illegible]

monitoring an enzyme-mediated ligand binding event in a test mixture, the kit comprises a Raman-active label, a particular having a surface capable of inducing a surface-enhanced Raman light scattering, and a specific binding member for the analyte.

III. BRIEF DESCRIPTION OF THE DRAWINGS

potential water film, (b) 2.4-dimethyl-3,5-dichlorobenzoic acid (DMCA) monolayer, and (c) 2.4-dimethyl-3,5-dichlorobenzoic acid (DMCA) monolayer with respect to DNP molecules, in the presence of a chemically deposited silver film, and (c) 2,4-dimethyl-3,5-dichlorobenzoic acid (DMCA) monolayer with respect to DNP molecules, in the absence of a silver film (for reference). The monolayers were prepared by spin-coating expanded liquid relative to A and B to enhance features). Species acquisition conditions: acquisition time, 18 sec; power, 45 mW; excitation wavelength, 633 nm.

FIG. 3 is a SEPPS spectrum obtained from chemically deposited silver film incubated in (A) a 3 mM solution of HABA and (B) a 2.5×10^{-6} M solution of silver. The solution of silver was subsequently made 0.3 mM in HABA. No discernible spectrum was observed in this region from surface silver deposited in the absence of HABA (C). Spectra are

[0044] FIG. 4 is a combined plot of typical SE-PMMA excitation wavelength, 457.9 nm.

antibody conjugate (A) SEITPS spectrum of 40 μ g/ml solution of DAb-anti-TSH antibody conjugate in the absence of a silver surface. Plots (B), (C), (D), (E), and (F) show spectra obtained by incubating cap-

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[0040] FIG. 1 is a profilometer tracing of an intrinsically chemically deposited silver film surface.

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Two antibody-coated detection solutions containing 0, 0.4, 2.0 and 8.0 $\mu\text{g/l}$ of TBSH antigens, respectively, followed by binder to a 24-well plate of Dynal (see below) were used. TBSH antigen-coated TBSH antibody conjugates, 1410 and 1410-SEBS, were used at 1410 and 1410-SEBS dilutions of 1:1000 and 1:1000, respectively. For each concentration of antigen measured, the concentration of antibody (defined as TBSH antigen-coated antibody) was 1:1000. The average of two replicate determinations was used. Each data point represents the average of four determinations. The numbers in parentheses are the coefficients of variation (standard deviation/mean) for the coefficients of variation.

concentration of 1.58 antigen/massated. [0046] FIG. 7 is a SERS spectra using near IR excitation for (A) spectrum of a blank ether film determined separately and added to a solution state spectrum determined in the absence of a silver surface, of the p-dimethylamino-azobenzene borate serum conjugate at 2900 mg/ml, (B) spectrum obtained by immersing the blank

Figure 1 FIG. 6 shows a no-wash immunostain of [10C47] against standards of human chorionic gonadotropin (HCG) prepared in pig serum, using goat colloid, a crystal violet dye or reporter molecule, and a SERPIS/rapid plotter.

[0049] FIG. 9 shows a no-wash immunosorbent assay of human chorionic gonadotropin (hCG) prepared in human saliva using gold colloid, a cross-linked ovalbumin molecule, and a SERPIS molecule as a function of hCG concentration. FIG. 10 shows a no-wash immunosorbent assay of

Fig. 11 shows a no-wash detection of the inhibition of binding by free toxin, of bovine serum albumin, and of the toxin itself. The inhibition of binding by free toxin, of bovine serum albumin, and of the toxin itself, plotted as a function of bovine serum albumin concentration.

(dimethylphenylacetylene (DAP), and boron, (substitution of complete conjugation is their-BSA-DAB), to self-assemble-coated silver colloid, by a SEPRs randomly placed as a function of chain-BSA-DAB concentration) [2005] FIG. 12 shows surface-enhanced Raman scattering (SEPRS) spectra at 20:1 mixture of methylphen-

made either using (A) hydrogen and (B) chloride as the reducing agent.

IV DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS

[0032] As previously stated, the present invention works as follows. At the beginning of the process, the user provides assay materials, compositions and kits for the determination of the presence or amount of an analyte in a test sample by monitoring fluorescence and changes in the surface-enhanced Raman scattering spectrum of the surface-enhanced Raman scattering spectrum of the test material which comprises the test sample, a specific binding molecule, a fluorescent label, and a photonic band having a surface capable of inducing surface-enhanced Raman scattering. It is believed that the presence of an analyte in a test sample facilitates the appearance of an analyte in a diagnostic spectrum of the surface-enhanced Raman scattering spectrum of the test material which comprises the test sample, a specific binding molecule, a fluorescent label, and a photonic band having a surface capable of inducing surface-enhanced Raman scattering.

[0033] Before proceeding further with the description of various embodiments of the present invention, a number of terms will be defined.

DEFINITIONS

[0064] "Analyte," as used herein, is the substance to be detected in the test sample using the present invention. The analyte can be any substance for which there exists a naturally occurring specific binding member (e.g.,

g., an antibody) or for which a specific binding method can be prepared, and the analyte can bind to one or more specific binding members in an assay. "Analyte" also includes any antigenic substances, haptens, antibodies, and combinatorials thereof. The analyte can include a protein, a peptide, an amino acid, a carbohydrate, a hormone, a steroid, a vitamin, a drug including

substances.
[footnote] "Zweites-maltes", as used here, refers to a substance which cross reacts with an enzyme specific binding member although it may do so to a greater extent than the substrate.

isomer, except that does the enantiomer itself. The enantiomer can "brouce a modified anlyse as well as a flagmented or synthetized portion of the anlyse molecule along as the anlyse anlyse has at least one eplopic element in common with the anlyse of interest.

[0035] "Anlyse applications" as used herein, denotes the part of the anlyse which consists one member of the

specific ligand among pair during the specific union event. That part of the specific binding pair member which contacts the epitope of the antibody during the specific binding event is termed the "epitope".

106571 *Mediator-mediated ligand binding event*, a used herein, means a specific binding event between two members of a specific ligand binding pair, the entire

amount present, of the analyte. This influence usually occurs because the analyte contains a structure, a *apofluor*, similar to or identical to the structure or apofluor contained by one member of the specific ligand binder

area. Unlike fluorescent readout systems, SEFS reports on groups will not self-quench so the signal can be enhanced by increasing the number of FRET pairs on the probe molecule. Fluorescent molecules near the SEFS active surface will actually be surface quenched.

7. Implementation

[0087] The present invention is adaptable for use with an automatic analyzer. Since the instrument would monitor discrete Stokes shifted spectral lines, the need for an absolute monochromator system is not necessary. Recent advances in stable-of-the-art optical technology, such as holographic optical elements, allow the design

of a suitable spectrometer with cost and complexity below that of the laboratory grade device.

[0095] Optical readout enables as a result of SEFS

are above that which require ultra-sensitive photon counting devices. In fact, some SEFES spectrometers now in use incorporate silicon photodiode detectors. The overall efficiency of a Microal manufacturing plant

In laboratory grade spectrometer it less than 1%. The advances in optical materials and components mentioned above would make possible two to three-fold im-

crates in optical efficiency for a simple spectrometer dedicated to only a few specific spectral lines. This also addresses one of the previously major concerns, blockage of the Rutherford scattering line. With Nitroflex annealing, the Rutherford line is clearly visible in the spectrum.

blities of newer filters on the order of 10^{-3} , substitution of three for one or more stages of the typical mono chromatizer system should be possible with significant

ccoll 1000/yr.

[0069] The general technology for analyzing an array in a test sample by means of a chromosome/phenotype array is known in the art. For example, Devast birching assay is known in the art.

at el. detect the chromatographic test strip device in U.S. Pat. Nos. 4,064,647, 4,235,601 and 4,361,937. These references are herein incorporated by reference. With

"Enzyme immunoassay: A quantitative immunoassay requiring no instrumentation," *Clin Chem*

31, 1144, 1985, further describe the assay principle. A so of interest are U.S. Patent Nos. 4,298,558; 4,517,289; 4,740,469, and 4,306,241; EP Publication

8 EXAMPLES

Example 1

[01:00] Support surfaces - Supports for the silver firm

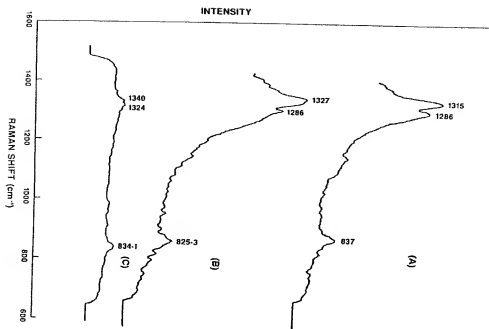


FIG. 2

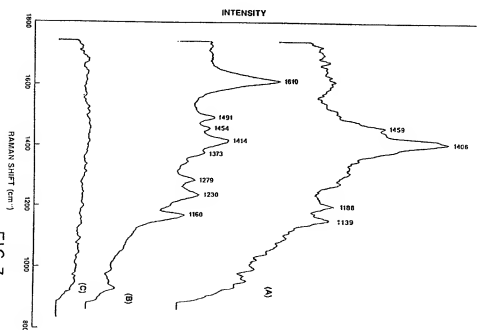


FIG. 3

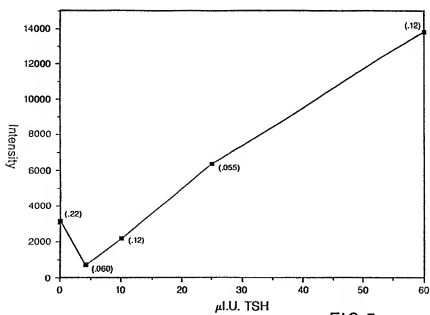
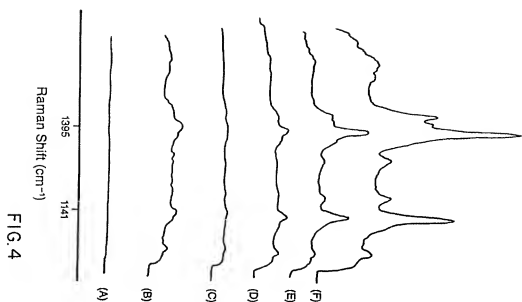


FIG. 5

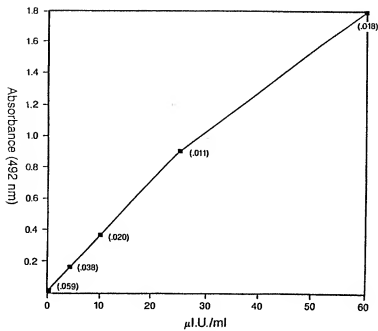


FIG. 6

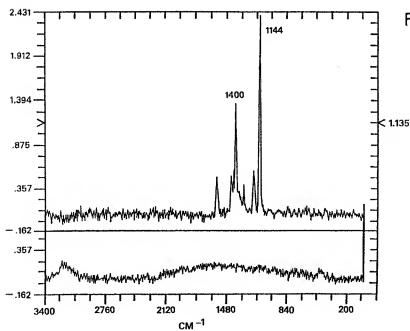


FIG. 7

FIG. 8

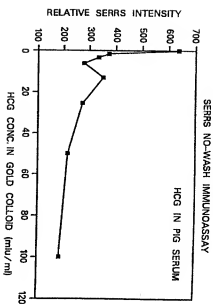


FIG. 10

